STUDIES IN EARTHWORM CHLORAGOGUE.

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The dearth of knowledge concerning the physiological significance of earthworm chloragogue and the opportunity offered for the study of the subject, are the main reasons for the work here presented, which has been carried on under the direction of Professor James G. Needham.

I have found only one article having an important bearing on the subject of this paper, one by Thomas Schaeppi,[1] which was found after this research was practically completed. It may be stated that in several respects a marked similarity exists between the chloragogue of *Ophelia radiata* and that of the earthworm, especially as to color, strong resistance to acid and alkaline reagents and the probability that the chloragogue is excretory in function rather than secretory.

Lumbricus herculeus has served for the most part as material for study. As much as was possible of the work was done with freshly collected material. But when, during the winter months, fresh material was not available, worms were used which had been collected in the late fall and, to insure a thrifty condition, preserved alive for winter use under favorable conditions of temperature and moisture. The worms were found to thrive best in temperature varying from 40° to 60° F. Extremes of heat were quickly fatal. It was found necessary to exercise care in the amount of moisture furnished, as too much moisture was as hurtful as too little. The results obtained from material properly preserved and from freshly collected material were essentially the same.

The following points are considered: I. Origin and growth of chloragogue; II. Distribution; III. Structure; IV. Function and V. Elimination.

I. *Origin and Growth.*—To learn whether the chloragogue, as is generally supposed, is a modification of the peritoneal epithelium, a series of worms was prepared beginning with a young

[1] "Das Chloragogen von Ophelia radiata" Jenaische Zeitschrift für Naturwissenschaft, 1893, XXVIII., Neue Folge XXI.

worm 5 mm. long and continuing through increasing sizes till a stage was reached in which the chloragogue cells appeared fully developed. In the smallest worms the peritoneal layer, the chloragogue that is to be, is always apparent and a similarity is evident between it and the peritoneal layer elsewhere, both in structure and in reaction to staining fluids. In the smallest worms the chloragogue appears as a simple layer as yet undifferentiated into the club-shaped cells of the adult form. In larger specimens of the series a gradual development into the typical chloragogue cells can be traced. As the cells become older they increase in length, the characteristic granules appear and they become less and less responsive to stains, until in the fully developed adult condition they are practically proof, excepting their nuclei, against every stain to which they were subjected. The nuclei readily respond to stain.

Upon adult worms the following experiment was performed to determine whether there was any renewal of chloragogue when it is artificially removed. With a sharp razor an incision was made through the outer body wall into the cœlomic cavity, exposing the alimentary wall. With a scalpel the chloragogue was then removed from the alimentary wall as completely as possible and the worm afterward kept under favorable conditions of temperature, moisture, etc., in order to insure a condition of thrift. The result of this experiment was that although the wound made in the body wall in order to remove the chloragogue began to heal well the second day after it was made, no chloragogue regeneration was observed in specimens sectioned at various intervals thereafter.

II. *Distribution.*—In the adult worm the chloragogue is found to be distributed as follows: beginning at the posterior end of the œsophagus it extends dorsally, attached in abundance to either side of the dorsal blood vessel as far back as the thirtieth segment (counting from the rear). It is interesting to note that the chloragogue attached to the outside of the alimentary canal *practically* ends in the region where the typhlosole ends: from the thirtieth segment to the anus it becomes less and less abundant, finally disappearing about the seventh somite. In the typhlosole it is also abundant. Somewhat less abundant than

on either side of the dorsal blood vessel, the chloragogue is found surrounding the origin of the aortic arches dorsally, and covering the dorso-intestinal blood vessels, an important fact, inasmuch as the blood gathers from the alimentary wall into the dorso-intestinal blood vessels and through them flows into the dorsal blood vessel. This condition suggests that the chloragogue may with good reason be considered as having to do with food elaboration. The chloragogue is found less abundant still on the outer surface of the alimentary wall, arranged in irregular circular bands. It is also found laterally and ventrally but not as abundantly as on the dorsal aspect. It is entirely absent from the ventral blood vessels, in marked contrast to its abundance on the dorsal vessels. In passing, reference may be made to the fact that within the dorsal blood vessel as seen in cross section a tissue is found, which in appearance, is much like the chloragogue. The largest individual chloragogue cells are found on either side of the dorsal vessel as it crosses the crop and gizzard. The walls of the crop and gizzard are free from chloragogue.

III. *Structure.*—The typical adult chloragogue cell is club-shaped, having at its narrow base a nucleus of medium size which readily stains, while filling the remaining cell space are innumerable minute yellow granules which compose the greater part of the cell and give to it its characteristic color, which varies from a brownish-yellow to a yellowish-green. These chloragogue granules are apparently lifeless. No stain has been found to affect them, the strongest acids like nitric acid and hydrochloric acid affect them but slowly. Likewise strong alkalies like potassium hydroxide have only a very slow effect in disintegrating them. Neither does feeding or starving a worm, as will be shown later, have any apparent effect upon the chloragogue. In the very young worm, however, the entire chloragogue cell is more or less responsive to stains, and the younger the chloragogue the more striking is its likeness to the peritoneal epithelial layer of which it is generally supposed to be a modification.

IV. *Function.*—One of the first attempts to determine the function of the chloragogue was through special feeding. A long series of negative results was obtained. The difficulty was not

found in the worms refusing to eat, because a few hours (over night) sufficed for a worm to gorge itself with whatever substance was placed before it even though it were no more than common white blotting paper. The worm, when the proper amount of moisture and the right limits of temperature were provided, thrived as well on fuchsin-stained earth ; on milk-saturated earth;[1] on finely divided meat scraps ; on moist bread ; on vegetables such as finely chopped leaves of cabbage, lettuce, etc., as it did on its customary earth diet. But no effect due to this varied feeding was observed on the chloragogue. This being so it was determined to try and affect the chloragogue through starving. A number of large thrifty worms were deprived of both food and water for as long a time as they could be kept alive, which was three days. In this time they lost 44 per cent. in weight, but a microscopical study in cross section of a worm thus treated, while it showed a great shrinkage in the cells of other tissues, did not show the chloragogue as having undergone any essential change. It cannot here be said that the function of the chloragogue has been definitely learned ; but the following inferences in the light of the foregoing and what is to follow seem justified. Finding the chloragogue in such close proximity to that part of the blood system which transports the newly absorbed food (see Fig. 1)—dorsal blood vessel, dorso-intestinal blood vessels and aortic arches—and within the typhlosole, and ceasing to be abundant on the outside of the alimentary wall at the point where the typhlosole ends within the alimentary canal, would indicate that the chloragogue had an important relation to the elaboration of food. But the lifeless condition of the adult chloragogue and its apparently living condition in the very young worm, indicate that possibly the function of the chloragogue is performed in the early development of the worm, and that in the adult it has become functionless, although in the

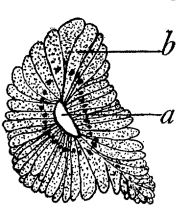

FIG. 1. Showing the characteristic abundance of chloragogue surrounding the dorso-intestinal blood vessels. *a*, dorso-intestinal blood vessel with (*b*) chloragogue cells surrounding it.

[1] Here it may be added that in histological preparations of milk-fed worms, treated with osmic acid, the characteristic black-colored fat globules could be distinguished in the epithelial cells of the alimentary wall but no trace of them was found in the chloragogue cells.

light of what follows the inference seems justified that it may have an excretory function.

V. *Elimination.*—Chloragogue granules are found free in the cœlomic fluid. They are found imbedded in leucocyte bodies. They are found composing the greater part of large waste masses,

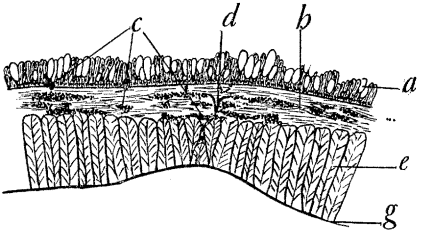

FIG. 2. Transverse view of median dorsal section of outer body wall showing characteristic elimination of minute waste masses of chloragogue granules. *a*, epithelial cells (hypodermis); *b*, circular layer of muscles; *c*, minute masses of waste chloragogue granules making their way to the outside dorsally. In Fig. 3 the masses are large but less numerous; *d*, nerve; *e*, longitudinal muscle layer; *g*, peritoneal epithelium of which the chloragogue is a modification.

in some instances filling the entire cœlomic cavity in the posterior region of the body, between numbers of the dissepiments. Lastly they are found in the muscular tissue of the outer body wall, dorsally and ventrally (see Figs. 2 and 3): on the dorsal

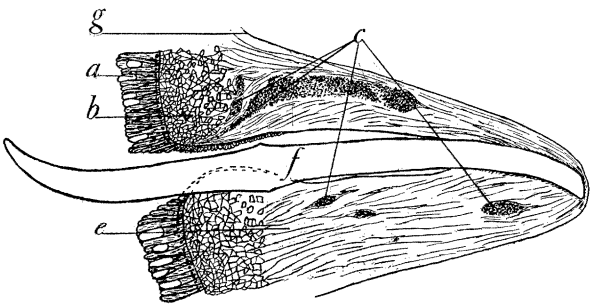

FIG. 3. Longitudinal view of portion of the outer body wall at the level of the ventral row of setæ showing waste elimination ventrally, of waste chloragogue granules in considerable mass. Letters signify same as in Fig. 2 with addition of *f*, seta.

aspect, for the most part as diffuse granular masses distributed throughout the circular muscular layer; on the ventral aspect in more compact masses in the vicinity of the setæ. They appear to be thus making their way to the outside. That there is

in the earthworm such a crude method of waste elimination is further supported by the results of an experiment performed by a fellow student at Lake Forest College, Mr. John J. Jackson. His experiment was undertaken to learn how the earthworm eliminates a foreign substance injected into the cœlomic cavity. Lamp-black was the substance used on this occasion. Histological preparations of material thus treated showed the lamp-black evidently making its way through the tissues of the outer body wall to the outside in the manner described for the waste chloragogue granules. That the letter are thus directly extruded any one may easily demonstrate by the following experiment: A thrifty worm thoroughly washed is placed under a bell-jar and subjected to the influence of ether vapor. The mucus which the worm casts off as a result of this ether vapor irritation, on microscopical examination is seen to contain an abundance of chloragogue granules which are enveloped or embedded in a transparent substance—possibly the contents of a mucous or a leucocyte cell that soon bursts, allowing the granules to scatter in various directions.

In the waste masses, for the most part composed of chloragogue granules, found in the anal region are large numbers of setæ, in one adult worm as many as six hundred and twenty-five (counted in part and estimated) were present, varying in size from small undeveloped to large fully developed ones. The size of the setæ depends somewhat on the size of the worm—the fully developed setæ naturally not being found in a very young worm while in an adult worm both very small and fully developed setæ are often present. Also in many of these same waste masses are found nematodes—*Anguilula lumbricei*, kindly determined for me by Dr. W. M. Woodworth. An interesting question arises in regard to the presence of these nematodes embedded in the waste masses in the posterior region of the body because of the following conditions. While some waste masses are entirely lacking in nematodes, such masses being of a dark brown color, other waste masses again are found which vary from a brown to an almost white color—depending on the number of nematodes present. If they are many, in which case the granules of chloragogue as a rule are scarce, the masses are white. The question

that has arisen from this condition of things is whether there may not be a symbiotic relations between the earthworm and these supposed parasites. Are these nematodes instrumental in consuming the chloragogue waste which the worm by other means is unable to eliminate from its body?

A detailed study of these waste masses both in preserved (for winter use) and freshly collected material gave these results: The average number of setæ (counted in part and estimated) present in the waste masses of each of the seventeen worms examined was two hundred and forty-eight. The nematodes in the dark-colored waste masses were as a rule few—sometimes entirely absent—and, when present, in a quiescent state, often enveloped by a sort of cyst. In these dark-colored masses the chloragogue granules far exceeded the nematodes in abundance.

In the light-colored masses, on the other hand, the nematodes were far in excess of the chloragogue granules, and on pressing the cover-slip many of the nematodes, having within their bodies granules apparently identical as to size and color with those of the surrounding waste chloragogue granules, could be excited to move freely and by so doing clearly showed themselves to be unencumbered by any cyst. Their appearance was suggestive of a newly moulted insect. This supposition of a symbiotic relation is then supported to the extent that within the nematodes have been found granules identical as to size and color with chloragogue granules; and further by the fact that where the nematodes are in excess and in an active condition the chloragogue granules as a rule are far less abundant than the nematodes. It should be stated, however, that the nematodes at no time have been seen in the act of consuming the chloragogue granules, though frequently one has patiently sought to observe this. Still the facts seem to justify the suggestion of a possible symbiotic relation existing between these nematodes and the earthworms within which they live.